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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of George E. Fox, Richard C. Willson III And Zhendong Zhang	
Serial No.: 10/057270	
Filed: January 26, 2002	Examiner
For: ...Methods for the Determination of Genetic Affinity of Microorganisms and Viruses	____________
Priority: Provisional 60/264,403 filed January 26, 2001	
Attorney Docket: ..010AUS	Art Unit ___
Response Due: None yet	

Invention Disclosure Statement

37 CFR 1.501

Assistant Commissioner for Patents
Box US; Group 1743
Washington, DC 20231
Sir:

In respect to the enclosed USPTO Form 1447, the following remarks are intended to distinguish the references known to the inventors.

The undersigned Attorney certifies that this Document has been filed in the U.S. Post Office via Express Mail ET601010116US addressed as above to the USPTO on 9 March 2002 *(37 CFR 1.10)*.

The Invention

This invention embodies selecting which sub-sequences in a database of nucleic acid such as 16S rRNA are highly characteristic of particular groupings of bacteria, microorganisms, fungi, etc. on a substantially phylogenetic tree. Also applicable to viruses comprising viral genomic RNA or DNA. A catalogue of highly characteristic sequences identified by this method is assembled to establish

the genetic affinity[1] of an unknown organism. The characteristic sequences are used to design nucleic acid hybridization probes that include the characteristic sequence or its complement , or are derived from one or more characteristic sequences. A plurality of these characteristic sequences is used in hybridization to determine the phylogenetic tree position of the organism(s) in a sample. Those target organisms represented in the original sequence database and sufficient characteristic sequences can identify to the species or subspecies level. Oligonucleotide arrays of many probes are especially preferred.. A hybridization signal can comprise fluorescence, chemiluminescence, or isotopic labeling, etc.; or sequences in a sample can be detected by direct means, e.g. mass spectrometry. The method's characteristic sequences can also be used to design specific PCR primers. The method uniquely identifies the phylogenetic affinity of an unknown organism without requiring prior knowledge of what is present in the sample. Even if the organism has not been previously encountered, the method still provides useful information about which phylogenetic tree bifurcation nodes encompass the organism.

The Prior Art

Prior Art will include the following References:

[1] "Genetic affinity" refers to how closely related two organisms are on a substantially phylogenetic tree. An organism has the highest genetic affinity to the smallest (i.e. most peripheral) cluster that encompasses it. On a substantially phylogenetic tree that is represented by signature probes in the assay, the invention determines what grouping encompasses an organism(s) in the test sample.

1. Fox, GE, Pechman, KR, and Woese, CR (1977) Comparative cataloging of 16S Ribosomal Ribonucleic Acid:Molecular Approach to Prokaryotic Systematics. Intn. J. Syst. Bacteriol. 27:44-57 (1977).

This paper describes methodology by which a bifurcating tree based on genetic affinity can be obtained from oligonucleotide sub-sequences of a large RNA. The subsequences are generated by a method known as oligonucleotide cataloging in which all fragments of an RNA ending in G but containing no internal G's are generated experimentally and individually sequenced. The method of the current invention does not seek to obtain trees of genetic affinity. Rather it utilizes the information contained in such trees to identify individual signature oligonucleotides that can be used in hybridization assays to determine the genetic affinity of organisms or viruses that may be present in a test sample.

2. Fox GE, Stackebrandt E, Hespel RB, Gibson J, Maniloff J, Dyer TA, Wolfe RS, Balch WE, Tanner RS, Magrum LJ, Zablen LB, Blakemore R, Gupta R, Bonen L, Lewis BJ, Stahl DA, Luehrsen KR, Chen KN, and Woese CR (1980) The phylogeny of prokaryotes. Science 209:457-463.

This paper describes the outcome of studies of genetic affinity among known organisms using partial sequence data from 16S ribosomal RNA that is generated by a method known as oligonucleotide cataloging. It was the first comprehensive tree published that displayed actual genetic affinity between most major bacterial groupings. The method of the invention does not seek to obtain trees of genetic affinity. Rather it utilizes the information contained in such trees to identify signature oligonucleotides that can be used in assays to determine the genetic affinity of organisms or viruses that may be present in a test sample.

3. Kohne, David E.; Method for detecting identifying and quantitating organisms and viruses; US Patent 5,288,611 granted 22 Feb 1994 which claims:

> "1. A method for detecting the presence of a species of organism comprising a ribosomal nucleic acid sequence, in a test sample, comprising the steps of: contacting ribosomal nucleic acid from said test sample with a nucleic acid probe able to hybridize to only a portion of said ribosomal nucleic acid sequence of said organism, incubating said probe and said ribosomal nucleic acid obtained from said test sample under specified."

This patent describes a method of using a nucleic acid probe targeted to ribosomal RNA in order to detect the presence of a specific targeted organism. The method of the invention seeks to determine the genetic affinity of whatever organism(s) is present in the test sample as closely as allowed by the probes included in the assay. When using the method of the present invention one need not know before hand which specific organisms might be present in the test sample and one can use target molecules other than ribosomal RNA. The method of the invention also provides a mechanism for identifying signature sequences that can be used to design suitable hybridization probes.

4. Kohne, David E.; Method for detection, identification and quantitation of non-viral organisms; US Patent 4,851,330 granted 25 July 1989 which claims:

"A method for detecting the presence in a test sample of any non-viral organisms belonging to a group, said group consisting of at least one but less than all non-viral organisms, which comprises: (a) bringing together any test sample rRNA and a nucleic acid probe, said probe having been selected to be sufficiently complementary to hybridize to one or more rRNA subunit subsequences that are specific to said group of non-viral organisms and to be shorter in length than the rRNA subunit to which said probe hybridizes; (b) incubating the probe and any test sample rRNA under specified hybridization conditions such that said probe hybridizes to the rRNA of said group of non-viral organisms and does not detectably hybridize to rRNA from other non-viral organisms; and, (c) assaying for hybridization of said probe to any test sample rRNA.

This patent describes a method of using a nucleic acid probe targeted to ribosomal RNA in order to detect the presence of a specific targeted group of organisms. The method of the 330 patent also utilizes a probe, which has been selected by differential hybridization. When using the method of the present invention one need not know before hand which specific group of organisms might be present in the test sample and one can use target molecules other than ribosomal RNA. Moreover, the method of the present invention seeks to determine genetic affinity not to detect a particular organism group. The method of the invention can be used with nucleic acids other than rRNA and also provides a mechanism for identifying signature sequences that can be used to design suitable hybridization probes.

5. Maidak BL, Cole JR, Lilburn TG, Parker CT Jr, Saxman PR, Stredwick JM, Garrity GM, Li B, Olsen GJ, Pramanik S, Schmidt TM, and Tiedje JM (2000) The RDP (Ribosomal Database Project) continues. Nucleic Acids Res. 28:173-174.

This paper describes a public database, which contains ribosomal RNA sequence information and bifurcating trees based on genetic affinity that can be calculated from that data. It also provides search tools that facilitate the design of hybridization probes that target specific organisms. This database is a very useful source of the type of information used to implement the method of the invention. It does not provide methods of identifying signature sequences needed to design signature probes and it does not describe methods for rapidly determining genetic affinity based on hybridization using signature probes.

6. McGill TR, Jurka J, Sobieski JM, Pickett MH, Woese CR, and Fox GE (1986) Characteristic archaebacterial 16S rRNA oligonucleotides. Syst. Appl. Microbiol. 7:194-197.

This paper describes a means of identifying ribonuclease T_1 signature sequences in oligonucleotide catalog data sets (lists of sequence subsequences consisting only of fragments of a ribosomal RNA ending in G which containing no internal G's). The approach described provides a formal way to define the ribonuclease T_1 signature sequences needed for an alternative method of evaluate higher-order branching possibilities generated by the usual clustering methods and to find placements for organisms that are not well handled by the usual clustering methods. Unlike the present invention, the method of this paper is not envisioned as a means to design probes that could be used in a rapid assay for genetic affinity. The current invention identifies signature sequences from any RNA or DNA without any of the sequence restrictions that apply to ribonuclease T_1 oligonucleotides and envisions the use of the resulting information as the basis for the design of hybridization probes. These probes can be used to determine the genetic affinity of organisms or

viruses containing the target RNA or DNA in a sample without prior knowledge of what organism or virus is likely to be present.

7. Uchida, T., Bonen, L., Schaup, HW, Lewis, BJ, Zablen, L., and Woese, C (1974) The use of ribonuclease U_2 in RNA Sequence Determination. J. Molec. Evol. 3:63-77.

This paper describes the experimental procedures and analysis methods used to obtain ribonuclease T_1 catalog data and specifically outlines the utility of using ribonuclease U_2 to accomplish this. The information in this paper is essential to understanding some of the other papers and patents that are referenced. Unlike the present invention, this paper does not describe methods for rapidly determining genetic affinity based on hybridization.

8. Woese CR (1987) Bacterial evolution. Microbiol. Rev. 51:221-271.

This review paper discusses in detail the genetic affinity of bacteria, and to limited extent eukaryotic organisms, as determined from 16S rRNA sequence and catalog data. It also introduces the idea of sequence signatures, which are "individual positions in the molecule, sequence signatures that define the various groupings". Examples of such individual signature positions that are highly characteristic of specific organisms groupings are given and used to evaluate the validity of the genetic affinities seen in phylogenetic trees constructed from 16S rRNA sequence data. The approach does not consider the quality of these individual position sequence signatures and it is not explained how one could obtain useful hybridization probes from just individual positions. Moreover, unlike the present

invention, this paper does not describe methods for rapidly determining genetic affinity based on hybridization.

9. Woese CR, Maniloff J, and Zablen LB (1980) Phylogenetic analysis of the mycoplasmas. Proc. Natl. Acad. Sci. USA 77:494-498.

This paper reports experimentally determined ribonuclease T_1 oligonucleotide catalog data for several organisms. The genetic affinity of these organisms is studied by a method called "signature analysis". According to this methodology "a set of oligonucleotides that is characteristic of (unique to) a group of organisms defines that group and is a "signature" for the group". This approach seeks to define genetic affinity of known organisms. The signature here is defined to be a collection of ribonuclease T_1 oligonucleotides that together define a group of organisms that have genetic affinity. The notion that a single oligonucleotide sequence could be used as a signature probe or to design signature probes is not considered. Unlike the present invention, the method of this paper is not envisioned as a means for rapidly determining the genetic affinity of an unknown organism. The current invention utilizes a substantially phylogenetic tree to identify individual signature oligonucleotides from any RNA or DNA without the sequence restrictions that apply to ribonuclease T_1 oligonucleotides and envisions the use of the resulting information as the basis for the design of hybridization probes that can be used to determine the genetic affinity of organisms or viruses containing the target RNA or DNA in a sample without prior knowledge of what organism or virus is likely to be present.

10. Woese CR, Stachebrandt E, Weisburg WG, Paster BJ, Madigan MT, Fowler VJ, Hahn CM, Blanz P, Gupta R, Nealson KH, and Fox GE (1984) The phylogeny of purple bacteria: the alpha subdivision. System. Appl. Microbiol. 5:315-326.

This paper applies the signature approach developed by Woese, Maniloff and Zablen (see above; Proc. Natl. Acad. Sci. USA 77:494-498)) to evaluate genetic affinities among various species of *Pseudomonas* deduced from ribonuclease T_1 oligonucleotide catalog data. The signature approach described by the authors is not envisioned as a method of designing hybridization probes or rapidly determining the genetic affinity of an unknown organism in a test sample. The current invention provides the means to identify individual signature oligonucleotides from any RNA or DNA without the sequence restrictions that apply to ribonuclease T_1 oligonucleotides and envisions the use of the resulting information as the basis for the design of hybridization probes that can be used to determine the genetic affinity of organisms or viruses containing the target RNA or DNA in a sample without prior knowledge of what organism or virus is likely to be present.

11. Woese; C. R., Stackebrandt, E., Macke, T. J., and Fox, G. E. "A Phylogenetic Definition of the Major Eubacterial Taxa", System Appl. Microbiol. 6: 143-151 (1985)

This paper describes methodology to display the distribution of any ribonuclease T1 oligonucleotide in a database of approximately 400 16S rRNA catalogs. This made it easier for the user of the graphical display to subjectively identify the ribonuclease T1 sequences that together formed the signature of a particular phylogenetic grouping. The method described does

not take advantage of a signature quality function and it is implemented for the purpose of defining genetic relationships among known organisms rather than identifying the genetic affinity of an unknown organism. The method of the current invention identifies signature sequences from any RNA or DNA without any sequence restriction and envisions the use of the resulting information as the basis for the design of hybridization probes.

Differences From Prior Art

The invention uniquely and rapidly identifies the phylogenetic affinity of an unknown organism without requiring prior knowledge of what is present in the sample. The invention is determining genetic affinity rather than detecting or identifying. This is a major distinction from Kohnne and similar methods.

Woese's "sequence signature" method does not consider the quality of the individual position sequence signatures and it does not teach how one could obtain useful hybridization probes from these one base sequence signatures. Woese's earlier "signature analysis" (including the McGill *et al.* refinements) focuses exclusively on ribonuclease T_1 oligonucleotides which have substantial sequence restrictions. Moreover, the purpose of this method was to either evaluate the quality of phylogenetic trees produced by methods such as cluster analysis and/or to idependent of such methods determine phylogenetic relationships. Unlike the present invention, Woese does not describe methods for rapidly determining genetic affinity based on hybridization. In contrast to Woese's method, the current invention does not seek to either make or evaluate a substantially phylogenetic

Rather, it uses the information on such a tree to determine signature sequences that will be useful in designing probes that can be used to rapidly determine genetic affinity.

There is a additional literature that we did not specifically cite because it is less relevant and relates to the use of RNA/RNA or RNA/DNA hybridization to determine genetic similarity between organisms. Such methods use whole rRNA as the probe or perhaps restriction fragments and certainly do not base their probes on an existing tree so they are less relevant to the present invention. Still other less relevant literature relates to direct sequencing or other methods of determining genetic affinity.

This invention offers several important improvements over the methods taught by the above prior art as it is understood:

A. Provides a method of rapidly determining the genetic affinity of previously totally unknown organisms.

B. Can provide information on the identitites of several organisms present in the same sample, even if none has been seen before.

C. Can be based on non-RNA, even non-nucleic acid signatures, or mixed signatures using information from nucleic acids, protein composition, phenotypic activities such as enzymatic activities.

Respectfully submitted,

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Attorney for Applicants
Registration No. 22,080

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Enclosure: Forms PTO/SB/42
Copies of References
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Substitute for form 1449A/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(use as many sheets as necessary)

Sheet 1 of 3

Complete if Known	
Application Number	10/057,270
Filing Date	26 Jan 2002
First Named Inventor	FOX
Art Unit	
Examiner Name	
Attorney Docket Number	010AUS

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number: Number - Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	3	US-5,288,611	22 Feb 1994	Kohne	
		US-			
		US-			
	4	US-4,851,330	25 July 1989	Kohne	
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FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document: Country Code[3] - Number[4] - Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]

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Sheet 2 of 3

Complete if Known	
Application Number	10/057270
Filing Date	26 Jan 2002
First Named Inventor	FOX
Group Art Unit	
Examiner Name	
Attorney Docket Number	D10AUS

OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
	1	FOX, Comparative cataloguing...Systemics Intn. J. Syst. Bacteriol 27, 44-57 (1977)	
	2	FOX, The phylogeny of prokaryotes Science 209, 457-463 (1980)	
	5	MAIDAK, The RDP... continues Nucleic Acids Res. 28, 173-174 (2000)	
	6	McGill et al, Characteristic... oligonucleotides Syst. Microbiol., 7, 194-197 (1986)	
	7	UCHIDA et al, The use of... Determination J. Molec Evol. 3, 63-77 (1974)	
	8	WOESE, Bacterial evolution ~~M~~ Microbiol. Rev. 51, 221-271 (1987)	
	9	WOESE, Phylogenetic... mycoplasmas Proc. Natl Acad. Sci. 77, 494-498	

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Sheet 3 of 3

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OTHER PRIOR ART – NON PATENT LITERATURE DOCUMENTS

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	10	WOESE et al, The phylogeny...subdivision Proc. Natl Acad. Sci. 77, 494-498 (1984)	
	11	WOESE et al, A phylogenetic...Taxa system Appl Microbiol, 6, 143-151 (1985)	

Examiner Signature		Date Considered	

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